Live Mammalian Cell Arrays

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Abstract: High-content assays have the potential to drastically increase throughput in cell biology and drug discovery, but handling and culturing large libraries of cells such as primary tumor or cancer cell lines requires expensive, dedicated robotic equipment. We have developed a simple, yet powerful method that uses contact spotting to generate highdensity nanowell arrays of live mammalian cells for the culture and interrogation of cell libraries.

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High-content assays have the potential to drastically increase throughput in cell biology and drug discovery, but handling and culturing large libraries of cells such as primary tumor or cancer cell lines requires expensive, dedicated robotic equipment. We have developed a simple, yet powerful method that uses contact spotting to generate high-density nanowell arrays of live mammalian cells for the culture and interrogation of cell libraries.

Cell-based assays and the tools used to perform them are constantly undergoing improvements toward higher experimental throughput, reduced reagent consumption, and advanced control of the cell microenvironment[1,2]. There are currently two main approaches for conducting high-content cell culture experiments. In the first, cells are cultured in microtiter plates and robotic equipment is used to perform all necessary fluidic operations[3]. Although effective, this method remains unavailable to many laboratories due to the requirement for expensive robotics. A second approach that can be used for high-throughput cell studies is reverse transfection[4-6]. Here, cells are seeded onto an array of DNA, RNA, or small molecules. Large gene expression and silencing studies can be conducted in this manner, but complex cell libraries cannot be investigated since each array is limited to a single cell type.

As an alternative to these approaches, contact spotting offers an affordable yet high-throughput platform. This technique is extensively used to generate high-density DNA and protein arrays and has been adapted to a variety of other purposes. For example, Hart *et al.* created a high-content immunoassay by spotting fixed mammalian cells that were cultured under different conditions[7]. To date, contact spotting has not been used to array live mammalian cells due to rapid spot evaporation and consequent cell death. Therefore, arraying of live mammalian cells has been limited to inkjet printing, which lacks the ability to handle a large number of different samples[8,9].

Large collections of mammalian cell lines have recently become available, including primary tumor and cancer cell lines[10], stably transfected expression cell lines, and GFP-fusion libraries[11]. Novel approaches are required to efficiently assemble complex arrays of hundreds to thousands of genetically diverse cells. To address this arising need, we developed a simple, fast, and scalable method that uses standard microarray printing tools to generate high-density nanowell arrays. A minimal sample requirement of 500 cells enables the interrogation of cells that are available in limited quantities or cells that cannot be expanded due to undesirable changes in phenotype or genotype, as in the case of stem cells and cancer cell lines[12].

We successfully generated live mammalian cell arrays (LMCAs) of both primary cells and commonly used cell lines. We harvested cells from standard culture formats and re-suspended them in Percoll, a high-density, cell-compatible colloidal suspension routinely used for isolation of primary cells. Cells plated in conical 384-well plates were taken up by a microarray spotting pin and delivered to a 48 well poly(dimethylsiloxane) (PDMS) array (~ 20 wells/cm^2) or a 675 well acrylic array (~ 36 wells/cm^2) adhered to a coverslip or glass slide, respectively (**Supplementary Fig. 1**). For comparison, the density of a 1,536 well plate is ~ 14 wells/cm^2. Each nanowell in the array has a 500 nl capacity and was filled with medium prior to spotting to ensure viability and to enable the automated handling of nanoliter volumes of live cells. Once arrayed, cells were incubated in a high humidity environment for 60 minutes to allow for attachment to the glass surface (**Supplementary Fig. 1**). After attachment, the array was covered with medium for

culturing and imaging (**Fig. 1a**).

We sought to improve high-throughput cell culture methods by drastically reducing the number of cells required for assay preparation. Arraying cells suspended in standard medium resulted in poor performance in terms of cell transfer, requiring hundreds of thousands of cells per sample (**Fig. 1b**). Re-suspending cells in Percoll improved the transfer efficiency by several orders of magnitude. This approach allowed spots containing ~40 cells to be reproducibly arrayed from samples of 1,000 cells (**Fig. 1b**). The number of cells delivered to each nanowell can be tuned by the initial sample cell density, so that between ~10 to 400 cells can be delivered from input samples of 500 to 50,000 cells (**Fig 1b**). The number of cells transferred decreased with successive printings, but it is nonetheless possible to print repeatedly from the same sample (**Supplementary Fig. 2**).

A critical aspect of any arraying technology is array integrity. Cross-contamination between nanowells can in principle occur by two mechanisms: i) carryover of cells between spots by the pin, and ii) direct transfer of cells between nanowells during medium addition to the nanowell array. To investigate contamination via carryover, we alternated between spotting cell and medium samples. No cells were found in the negative control nanowells, indicating that the pin washing procedure eliminated sample carryover (**Fig. 1c**). To investigate direct transfer of cells between nanowells during medium addition, we spotted an array of cells in which the majority of wells was left empty. These originally empty wells remained uncontaminated after medium exchange (**Fig. 1d**). When printing larger arrays where all nanowells were programmed with cells, a low degree of contamination was identified, amounting to 2 out of 48 nanowells (4%) (**Supplementary Fig. 3**).

To assess the viability of cells after the arraying process, we prepared 48 separate aliquots of NIH-3T3 fibroblasts on a source plate and transferred each to a separate nanowell. By washing the pin between each sample, we simulated the arraying of 48 different cell samples (**Fig. 1e**). The fibroblasts stably expressed a red fluorescent protein that rapidly disperses upon cell lysis, allowing fluorescence to serve as a direct measure of cell viability. Following arraying, the cells remained viable and quickly became confluent in the nanowell (**Supplementary Fig. 4**). We also tested the viability of LMCAs after freezing, as it would be convenient to generate many identical cell arrays in one printing session and use them sequentially at later time points. Both the 48 well PDMS and 675 well acrylic LMCAs could be frozen and thawed without substantial effects on cell viability (**Supplementary Fig. 5**). This approach may be useful for ultra-high density cryogenic storage of cell culture samples.

The LMCA method is generic and can be applied to a variety of mammalian cell types, including NIH-3T3 fibroblasts and other commonly used cell lines such as human liver carcinoma (Hep G2), Human Embryonic Kidney (HEK), and Chinese Hamster Ovary (CHO) cells (**Fig. 2a** and **Supplementary Fig. 6**). We also applied this method to primary cells. Human bone marrow derived mesenchymal stem cells (MSCs) were spotted and maintained in culture for over two weeks with no culture contamination or detectable decrease in viability (**Fig. 2a**), indicating that the LMCA approach is compatible with primary cells and long-term culturing assays.

To demonstrate the scalability of this technique and its potential for applications involving large cell libraries, we increased the original array size from 48 nanowells to an array of 675 nanowells (**Fig. 2b-c**). The 675 nanowell arrays are commercially available (ALine, Inc.), and dimensions such as well size and pitch can be customized. Our array featured wells of 1 mm diameter and 1.5 mm pitch, enabling the entire substrate to fit on a microscope slide. A single 4 well microtiter plate could thus be used to culture up to 2,700 cell types. We programmed 339 of the 675 wells with cells (**Fig. 2b**). 24 hours post-spotting, 331 of the wells contained viable cells, indicating a 2% failure rate in cell transfer. 40 wells that were not originally spotted were found to contain cells (32 containing a single cell, with the remainder containing two or three cells). Therefore, 7% (48 / 675) of the nanowells were either contaminated or contained no cells when they should. Since this contamination occurred during the media addition step, we anticipate that optimization of the

array and liquid-handling steps could further improve the performance of the 675 well array.

Although powerful on its own, LMCAs are compatible with other high-throughput approaches such as transfection. The standard reverse transfection protocol uses microarraying pins that generate small diameter spots (120-150 μm) that dry rapidly and uniformly[4]. However, for our LMCA nanowells, spots of 1 mm diameter were required. Larger spots tend to dry slowly and unevenly, causing the majority of the transfection reagent to concentrate at the edges of the nanowell. Reverse transfection has been successfully achieved using the large wells of 24-well microtiter plates[13], however the microliter volumes of reagents administered to each well preclude the use of a microarrayer. We developed a method that uses the microarraying pin for automated deposition of homogeneous lipid-DNA spots into the nanowells of our PDMS arrays. Upon introducing cells into the wells with a micropipette, we achieved 32% transfection efficiency, with transfected cells uniformly distributed throughout the nanowell (**Fig. 2d**). Manually depositing larger volumes of reagent did not further improve efficiency (**Supplementary Fig. 7**), suggesting that our spotting protocol was sufficient to increase the local concentration of the transfection reagent at the site of cell attachment. Our method addresses two limitations faced by standard reverse transfection; the physical separation of cells on our array prevents cross-contamination and also allows for the inclusion of additional soluble factors.

In addition to reverse transfection, we explored a regular transfection approach. In this method, we spotted cells prior to adding lipid-DNA complexes, which resulted in higher viability and control over cell density in comparison to our reverse transfection assays. Using the acrylic-based 675 well array, transfection efficiency was 20% for the wells with manually added transfection reagent (**Fig. 2e**). Manual submersion of an array into medium containing lipid-DNA complexes could be used to simultaneously transfect a variety of cell types. Efficiency was lower for wells in which the lipid-DNA was introduced by the microarraying pin (13%), most likely due to the limited ~20 nl transfer volume of the pin (**Supplementary Fig. 7**). In contrast to the acrylic 675 well array, we achieved considerably lower efficiency with the PDMS-based 48 well array (**Supplementary Fig. 7**).

The LMCA platform can be reliably used for a variety of cell types, making it ideal for screening assays. Despite their large size, MSCs could also be controllably spotted at different densities, exhibiting a trend similar to that observed with standard cell lines (**Fig. 3a**). We were able to spot ~5 cells into the nanowells when using a sample well containing as few as 500 cells, and up to ~80 cells when using inputs of 7,000 cells (**Supplementary Fig. 8**). We repeatedly used each sample well to spot four nanowells, resulting in fewer cells deposited into each subsequent nanowell. This procedure provides a simple way to achieve a variety of cell densities from a single sample.

To demonstrate the possibility to perform long-term assays with LMCAs, we investigated the adipogenic differentiation potential of MSCs cultured on arrays for up to 10 days. We spotted the MSCs at a variety of densities and incubated the array in proliferation medium for two days. The array was then placed into differentiation medium for eight days. At the end of the ten-day period, the array was fixed, stained with DAPI and Nile Red, and imaged. Wells that initially contained higher numbers of cells exhibited pronounced adipogenic differentiation, as measured by Nile Red staining (**Fig. 3b**, red and white pixels indicate highest differentiation). These cells produced large lipid vesicles that appeared as dark spots in brightfield. In contrast, wells that were seeded at lower densities contained cells that differentiated to a lesser extent and produced fewer and smaller lipid vesicles.

In summary, we generated high-density LMCAs with a variety of mammalian cell types, including primary stem cells using contact spotting. This simple yet powerful method requires only a standard contact microarrayer and a nanowell array that can be easily fabricated[14] or commercially acquired (ALine, Inc.). Different soluble factors can be tested on the same array due to the physical separation of the samples. In a humidified environment, the liquid contained in the nanowells can be maintained for 24 h. The entire array is easily imaged, enabling high-

content microscopy studies. In this work, LMCAs were combined with techniques such as transfection, cell fixation, and staining. The cell arrays could also be used in conjunction with arrays of hydrogels and biomolecules or for artificial extracellular matrix screening[15,16]. This technique will be useful for interrogating cells that are available in limited quantities, as arraying can be performed with as few as 500 cells. The high-throughput approach to cell handling described here is readily accessible and will thus enable the parallel culture and analysis of large libraries of mammalian cells.

Methods

Methods and any associated references are available in the online version of the paper.

Note: Supplementary Information is available in the online version of the paper.

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Author Contributions

LMF, KW, MPL, and SJM designed experiments and developed the method. LMF and KW performed experiments. SG assisted with cell culturing, image processing, and providing transfection reagents. LMF, KW, MPL, and SJM wrote the paper. SJM conceived the idea.

Competing Financial Interests

The authors declare no competing financial interests.

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Figure 1 Development of a method to array live mammalian cells. (**a**) Schematic of method. (**b**) Quantification of cell transfer. The number of cells transferred to a nanowell increases dramatically when cells are re-suspended in Percoll as compared to cell culture medium. Graphs show mean ± s.d. (**c**) Quantification of cell carry-over by the spotting pin. (**d**) Quantification of direct cell transfer across wells. Three wells were spotted with red 3T3 fibroblasts (first row) and three wells were spotted with green HEK293 cells (third row). No cells are observed in adjacent empty wells. (**e**) A 48 well array was seeded with red NIH-3T3 fibroblasts and imaged two days after spot- ting. Cells grew after spotting and quickly became confluent in the area defined by the PDMS membrane.

Figure 2 Applications of the arraying method. (**a**) Five different cell types were spotted and cultured in the nanowell arrays (NIH-3T3, Hep G2, HEK293, CHO, hMSCs). (**b**) 339 of the wells of a 675-well array were spotted with 3T3 fibroblasts by simultaneously using 3 spotting pins. The entire array is shown on the left, with a close-up image on the right. (**c**) An EPFL logo was spotted using the same approach. (**d**) Reverse transfection: a lipid-DNA mixture was spotted into the 48 nanowells of a PDMS array and allowed to dry, followed by manual addition of CHO cells to the array. The left panel shows transfected cells expressing tomato, and the right panel shows all cells in brightfield. (**e**) Regular transfection: CHO cells were spotted into the 675 well array, followed by spotting of transfection mixture into the wells. The left panel shows transfected cells expressing tomato, and the right panel shows all cells in brightfield.

Figure 3 Mesenchymal stem cell array. (**a**) hMSCs were spotted into the 48 well array from sample wells containing between 500 and 7,000 cells. DAPI and phalloidin stains were used to calculate cell numbers and visualize cell shape, respectively. (**b**) MSC array differentiation scheme and micrographs of individual nanowells from the array. DAPI and Nile Red stains were used to measure cell density and adipogenic differentiation, respectively. On the DAPI micrographs, cell numbers are indicated. In the Nile Red pseudocolor scale, the highest adipogenic differentiation is indicated by red and white pixels.

ONLINE METHODS

Substrate fabrication. PDMS membranes were molded from SU8 structures fabricated on silicon wafers using standard photolithography methods[17]. Two layers of approximately 200 µm thickness were spun and baked before exposure and development. Before use, the structures were silanized using 1H,1H,2H,2H-perfluorooctyl trichloro silane (Sigma) as previously described[18]. The wafers were spin coated with PDMS (RTV615, General electric, 60 s at 150 rpm and 120 s at 350 rpm). The coated wafer was degassed for 5 min and left to re-flow for 25 min at room temperature before baking for 30 min at 80°C. This process resulted in a perforated membrane of approximately 200 µm thickness. The membrane featured 48 wells of 1 mm of diameter with a pitch of 2.25 mm.

The patterned PDMS was cut from the wafer and transferred to glass microscope coverslips. Separately, an array of BSA that matched the pitch of the membrane was spotted onto an epoxy-covered microscope slide. The membrane wells on the coverslip were manually aligned to the BSA spots on the slide using a stereoscope, providing a simple method to ensure alignment between the arraying robot and the microfabricated substrate. For some samples, a PDMS frame surrounding the membrane was manually cut and bonded to the coverslip using oxygen plasma. This frame provided an efficient medium container around the membrane and enabled high resolution imaging of the coverslip-supported cell array. Samples were UV-sterilized after assembly and coated with fibronectin (Sigma) either by spotting a concentrated suspension (1 mg/ml) or by 1 h incubation in a dilute solution (50 µg/ml).

The 675-well array plastic substrates were purchased from ALine, Inc. The arrays consisted of a 500 µm acrylic layer and a 50 µm silicone adhesive layer. The array featured wells of 1 mm diameter with 1.5 mm pitch. The arrays were adhered to glass microscope slides by passing them slowly through lamination rollers at 60°C. Arrays were not treated with fibronectin prior to cell spotting.

Cell culture. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin at 37°C and 5% CO_2. For long-term assays, medium supplemented with antibiotic-antimycotic was used. Cells were passaged every two days using TrypleE express or Trypsin. All culture reagents were acquired from Gibco (Life Technologies). NIH-3T3 fibroblasts stably expressed GFP or tomato fluorescent protein, whereas CHO and HEK293 cells expressed GFP (100% and 50% cells positive respectively). Other cell lines used included Hep G2, human MSCs, and wild-type CHO cells.

Cell spotting. Cells were harvested from T75 or T25 flasks and washed. After cells were adjusted to the desired concentration, they were transferred to 500 µl Eppendorf tubes in 20 µl aliquots. These aliquots were centrifuged at 300g for 3 min and re-suspended in a Percoll standard solution (700 µl Percoll (Sigma), 100 µl 10x Phosphate Buffer Saline (Gibco), 200 µl MiliQ water). For longer spotting programs, 10x PBS was replaced with 10x DMEM. Cells re-suspended in Percoll were plated in 5 µl aliquots onto conical-well, poly(propylene) 384-well plates (Arrayit). Cells were spotted using a pin with a 100 µm wide channel, a 1.25 µl uptake volume and a nominal 1.5 nl transfer volume (WCMP, Arrayit). Spotting parameters were 2 x 1 s inking time, 2 x 1 s print time per well. The washing protocol consisted of four alternating washes with ethanol and water. The first three washes were 2 s long and the last wash was 5 s long. 5 s drying was implemented following the last wash. Including spotting and washing, the total time per sample was approximately 30 s and therefore an array of 48 different samples was completed in under 25 minutes. Before spotting, the 48-well PDMS arrays were pre-filled by manual pipetting approximately 0.5 µl of 0.7x culture medium into each well. For the 675-well arrays, wells were pre-filled by submerging the entire array in 0.7x medium. Air bubbles were removed from wells by pipetting. The array was then slowly removed from the medium and excess liquid was wiped from the surface. The same printing conditions used for the 48 well array were used for the 675 well array, but only one 1.5 s water wash with 3 s drying was implemented. During spotting, the humidity in the chamber was set to 70% to reduce medium evaporation from the wells without

causing the printing malfunction that is observed at higher values.

Array culture. Immediately after spotting, substrates were placed in a petri dish that contained warm MiliQ water to prevent evaporation during transport to the incubator and during cell attachment. To preserve the integrity of the array, the samples were placed on a PDMS block adhered at the center of the dish, ensuring that the water contained in the dish did not come in contact with the sample. After incubation at 37°C (1 h for the 48-well array and 3 h for the 675-well array) the cell arrays were covered in medium for culture. To this end, either the coverslip was detached from the epoxy slide and placed in a different petri dish or, for samples featuring a PDMS frame, medium was directly added to the array.

To prepare arrays for freezing, LMCAs were placed in small plastic containers containing 90% FBS and 10% DMSO. The container was then placed into a cryobox with isopropanol and frozen at -80°C overnight. The arrays were thawed by submersion in a beaker of PBS contained in a 37°C water bath. Once thawed, arrays were washed 3 times in culture medium to remove residual DMSO.

For primary cell arrays, hMSCs were spotted onto a 48 well PDMS array following the procedure used for other cell types. MSC proliferation medium consisted of α-minimum essential medium (Invitrogen), 10% fetal bovine serum (FBS) (Hyclone), 1 ng/ml fibroblast growth factor 2 (FGF2; R&D Systems), 2 mM L-glutamine (Sigma-Aldrich), and 1% penicillin/streptomycin (Invitrogen). Adipogenic differentiation medium contained low-glucose Dulbecco's modified Eagle medium (DMEM; Invitrogen), 20% FCS (Hyclone), 0.5 mM 3-isobutyl-1-methylxanthine (IBMX) (Sigma-Aldrich), 60 µM indomethacin (Fluka) and 1 µM dexmethasone (Sigma-Aldrich). The arrays were fixed in 4% paraformaldehyde for 15 min at room temperature. The PDMS membrane was removed prior to cell staining. Nuclei were stained with a 10 µg/ml solution of DAPI (Sigma-Aldrich) in PBS. Lipid vesicles were stained with a 1 µg/ml solution of Nile Red (Sigma-Aldrich) in PBS. The cell cytoskeleton was visualized by staining with phalloidin-Alexa Fluor 488 (Invitrogen) according to the manufacturer's instructions.

Transfection. For the reverse transfection approach, glass slides coated with Poly-L-Lysine (Electron Microscopy Sciences) were used. 0.37 µl DNA (at 1.8 µg/µl) was diluted into 11.13 µl of a dextran solution. 2 µl Fugene (Roche) was added and the mixture was incubated for 15 min. 46 µl polyvinyl alcohol (PVA) was then added and the mixture was spotted into the nanowells using a pin with a 500 µm wide channel, a 1.25 µl uptake volume and a 15-25 nl transfer volume (WCMPL, Arrayit). Arrays were allowed to dry, after which cells were introduced into the nanowells by manual pipetting. Spotting conditions for the lipid-DNA mixture were identical to those used for cells except that only one water wash (1 s) with 3 s drying was used between samples, and 3 stamps per well were implemented. Dextran (M_r ~40,000, Sigma) was prepared at 45 mg/ml and PVA (MW ~25,000, Polysciences) was prepared at 0.5% (v/v) as described in Fujita *et al.*[13]. For regular transfection, cells were spotted as previously described. 2 h following spotting, a lipid-DNA mixture was prepared and spotted into the nanowells as described above, with the modification that fibronectin (1 mg/ml) was used in the place of PVA. Regular transfection arrays were not submerged in medium until 20 h after initial cell spotting.

Array Imaging. Imaging of the 48 nanowell array was performed on a Nikon Ti automated microscope using custom-written software to determine the positions to image trigonometrically and acquire the multichannel images. Images were typically acquired using 10x magnification to capture an entire well in the field of view. Array images were collaged using ImageJ. ImageJ was also used for cell counting. First, images where thresholded and inverted. Then, single cells were counted using the particle analysis functions. Cells that were aggregated or too close for direct counting were counted by measuring their area and calculating the number using a proportion obtained from manual counting of a group of cells from the same sample. DAPI-stained MSCs were counted using the count nuclei program of Metamorph.

Imaging of the 675 nanowell array was performed on a Nikon TI automated microscope using NIS

Elements. Each well was imaged with a 4x objective in brightfield and fluorescence mode. The resulting 675 images were first brightness adjusted and converted to jpeg from tiff format, then stitched together using the Grid/Collection Stitching plugin in Fiji[19].

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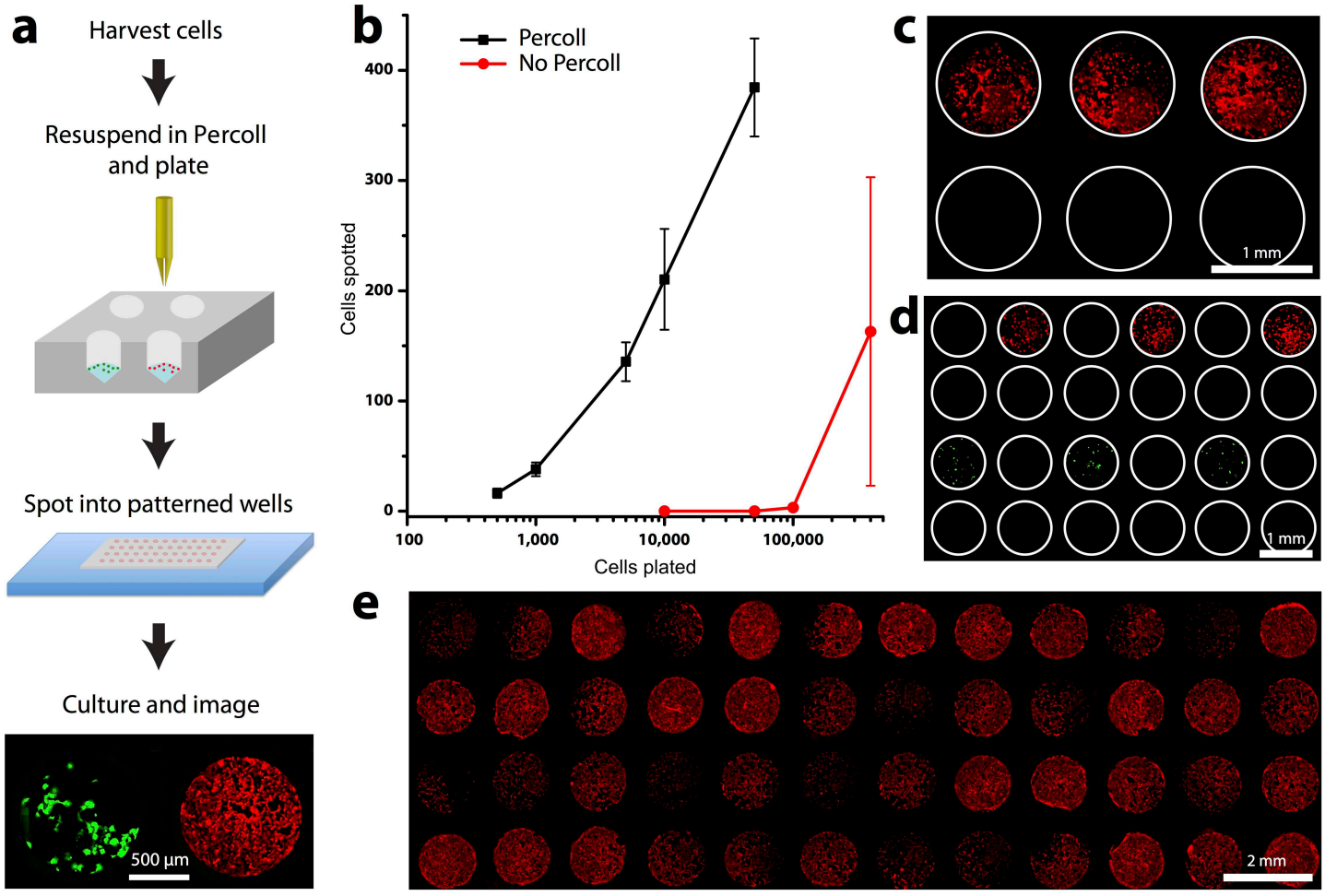
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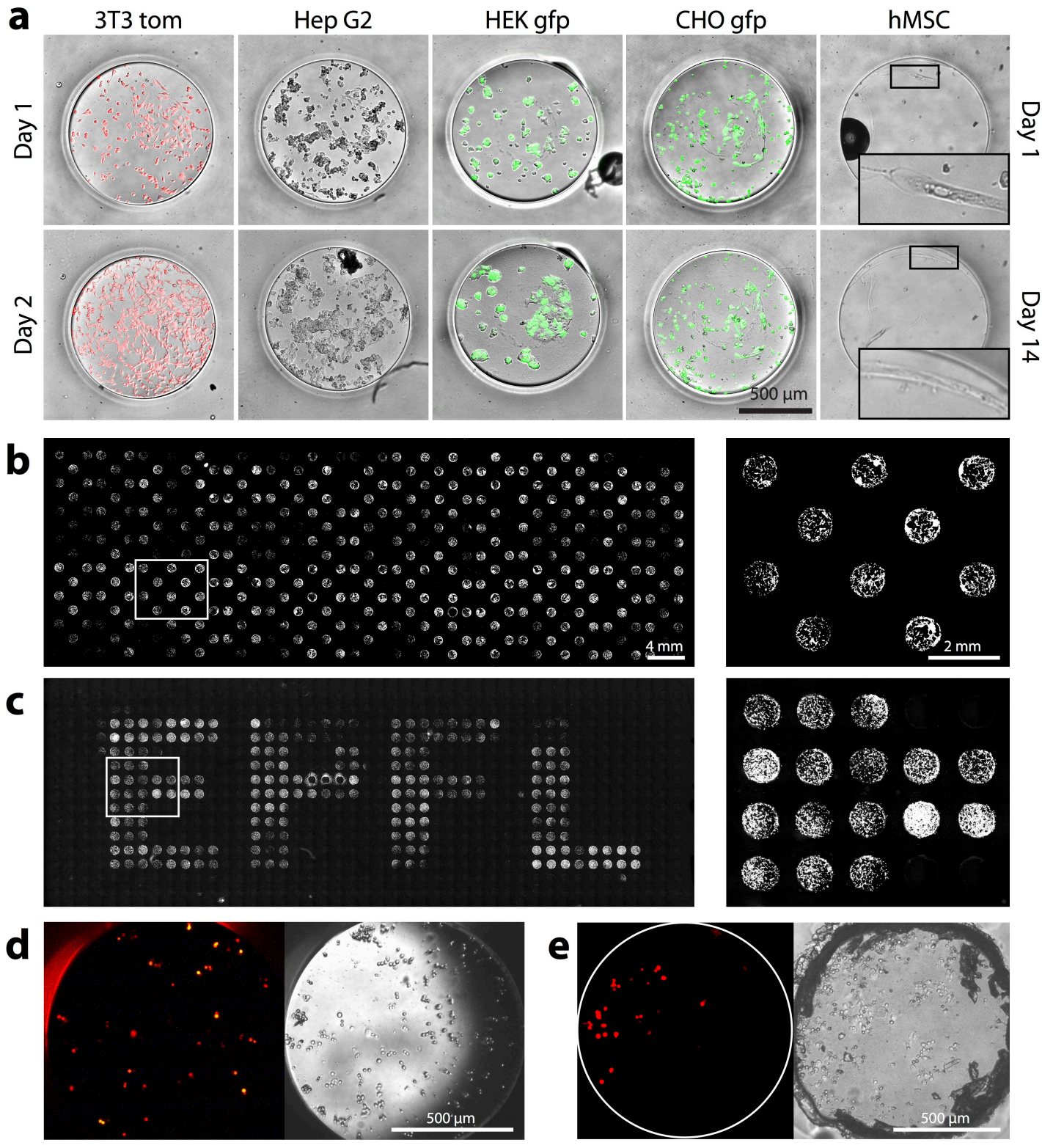
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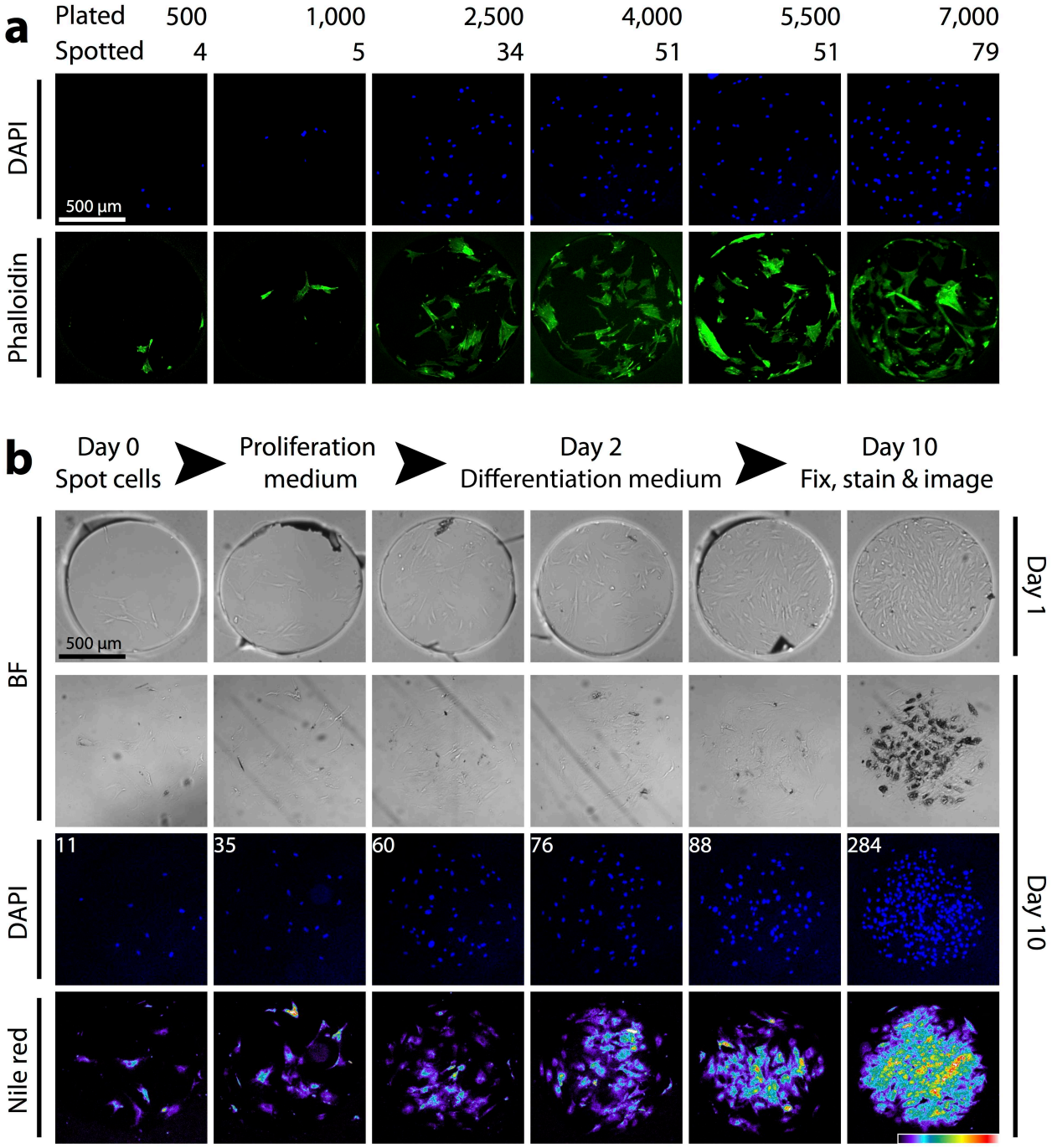
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Supplementary Information

Live Mammalian Cell Arrays

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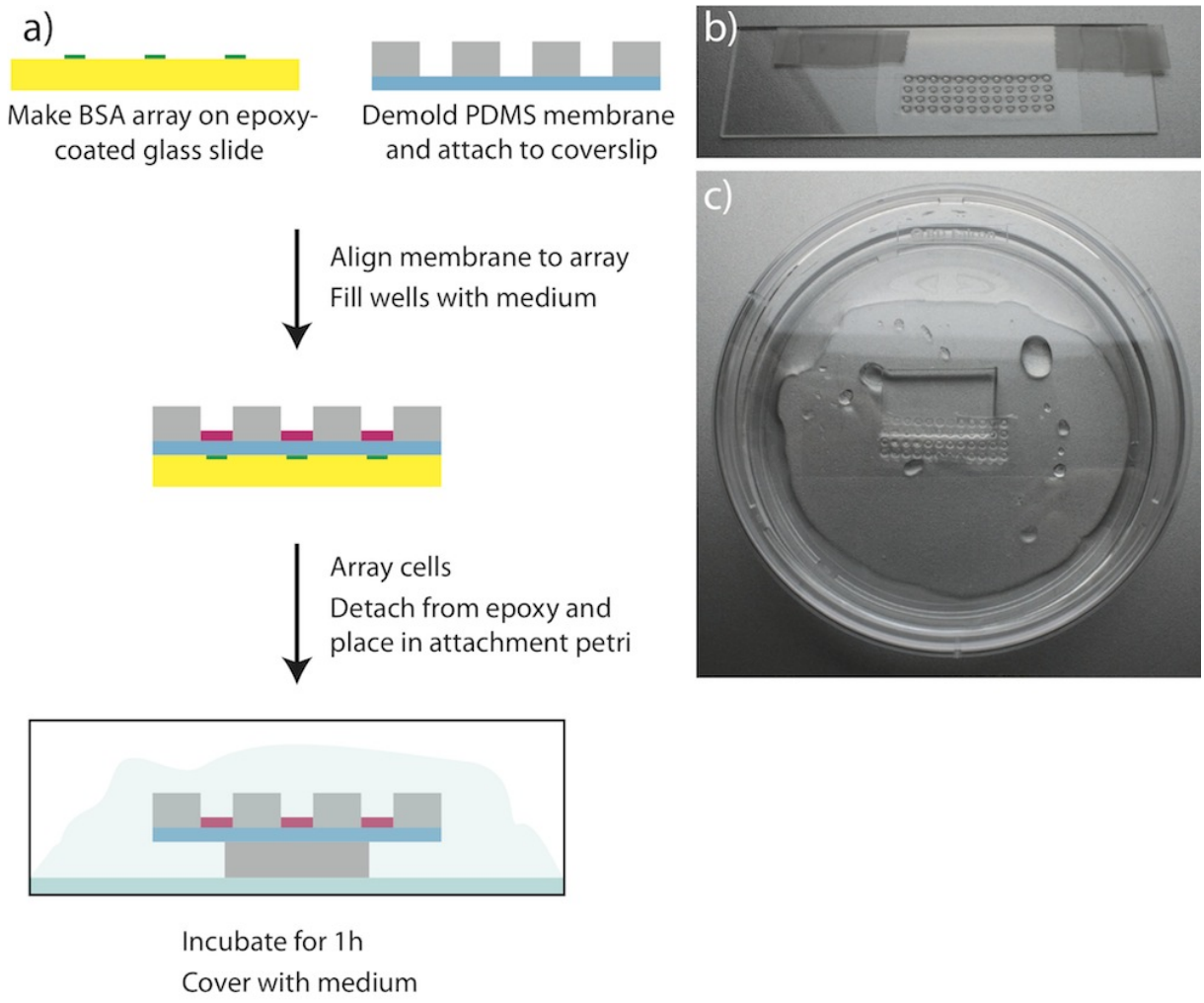
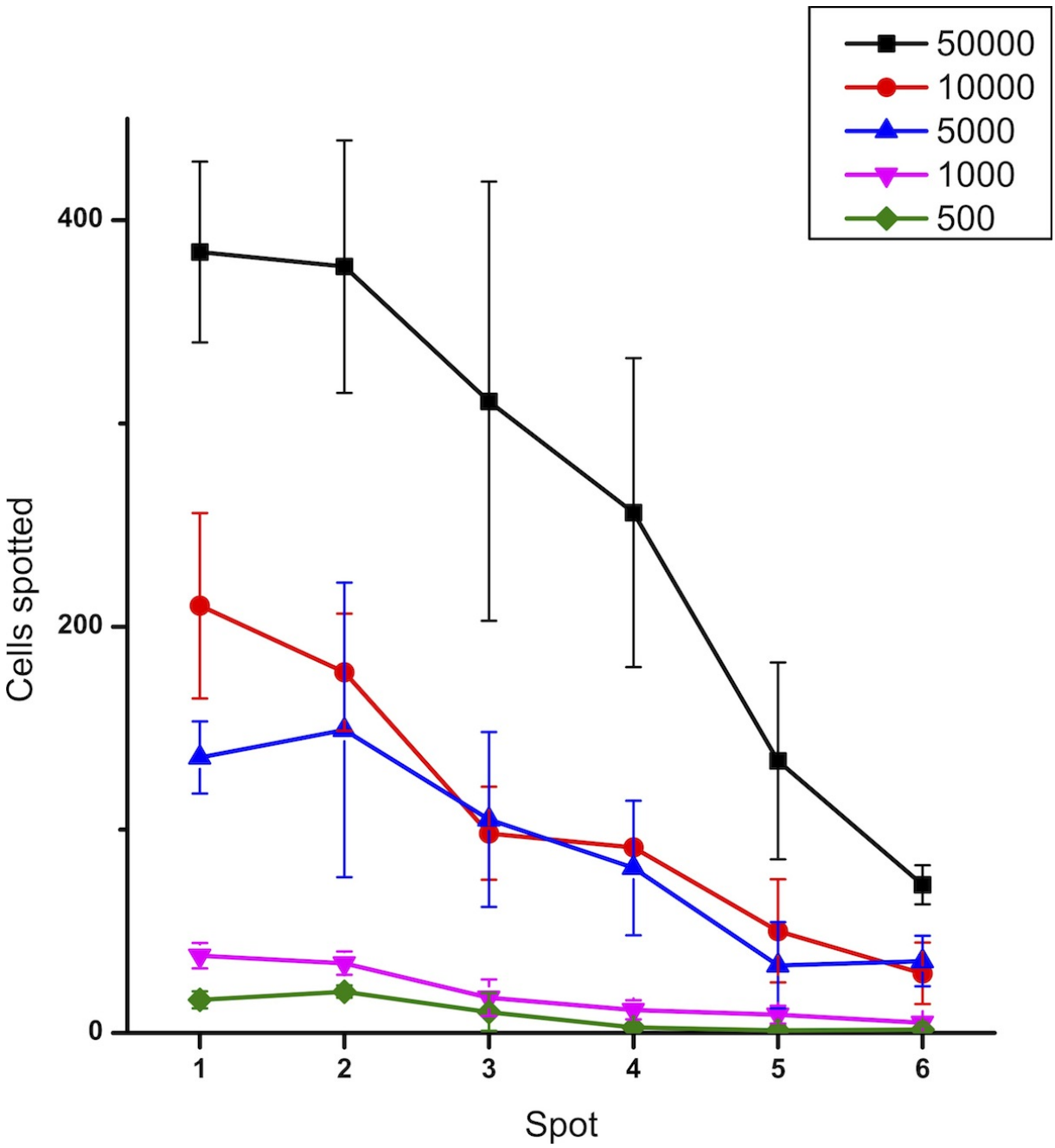
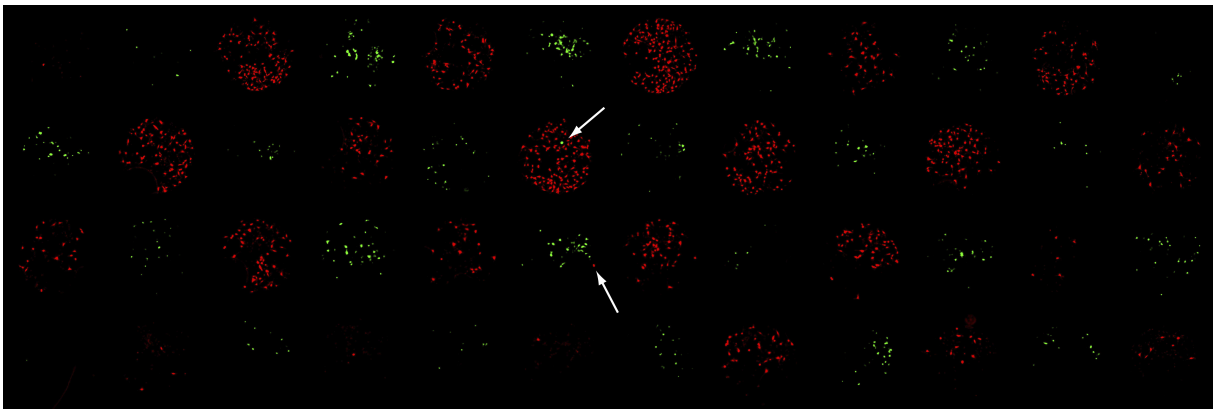


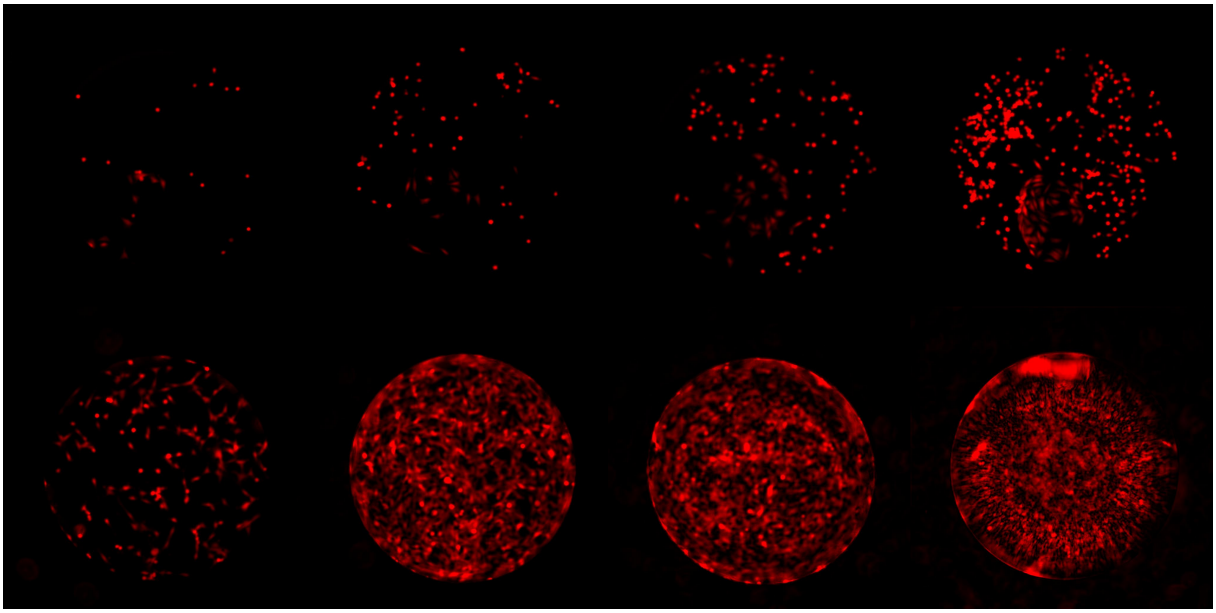
Supplementary Figure 1: **(a)** Schematic of substrate preparation and cell attachment. A perforated PDMS membrane of approximately 400 μm thickness was cut and adhered to a microscope coverslip. Separately, an array of 1% BSA matching the pitch of the membrane was spotted onto a microscope slide. The membrane wells on the coverslip were manually aligned to the BSA spots on the slide. The nanowells were filled with medium before cell spotting. Spotted arrays were incubated for one hour in a humidified petri dish to allow for cell attachment. After incubation, the array was covered with medium for culture. **(b)** Photograph of the PDMS membrane, the coverslip, and the epoxy slide. **(c)** Photograph of an array inside a petri dish during the cell attachment stage. The water in the dish quickly evaporates, saturating the atmosphere in the petri dish and preventing evaporation from the nanowells while cells attach.



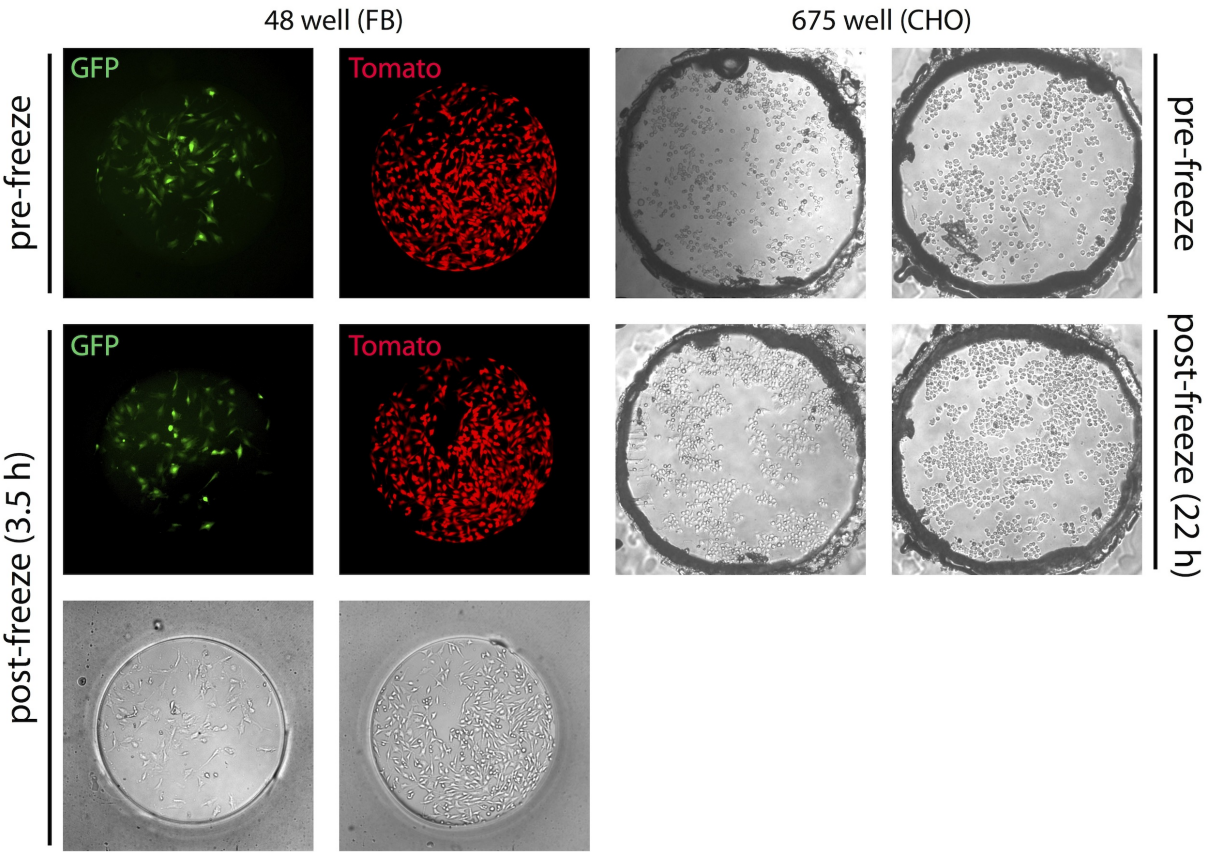
Supplementary Figure 2: Quantification of cell transfer vs. number of spots printed from the same well for five different cell densities. The number of cells transferred decreases as multiple spots are printed from the same well, but it is nevertheless possible to reliably print several samples using as few as 1,000 cells. Data points are the mean $\pm$ s.d. (n=3).



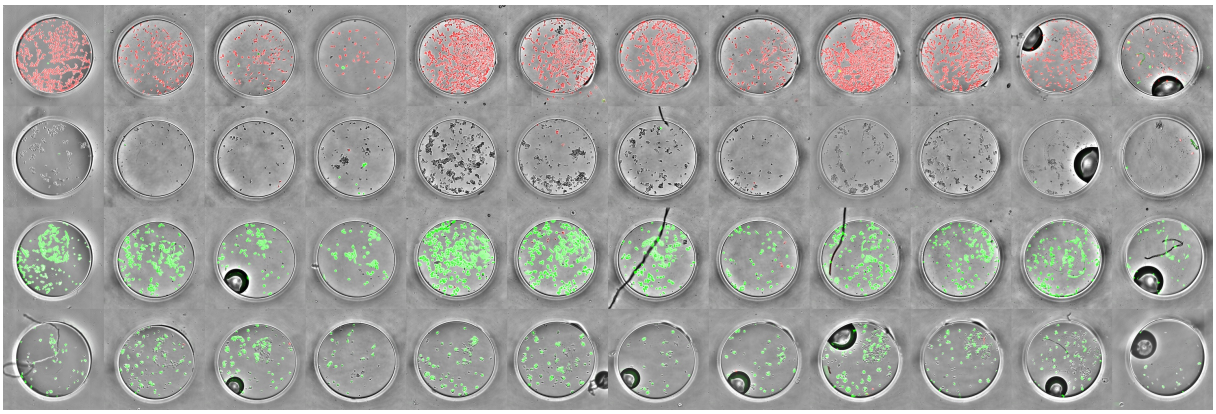
Supplementary Figure 3: Quantification of contamination due to direct transfer of cells across wells. Collage of micrographs of an array prepared with a checkerboard pattern of red and green fluorescent cells after one day of culture. The cells used were tomato NIH-3T3 and GFP HEK293. The array was prepared plating 48 wells with samples containing 5,000 cells each and spotting a single time from each of them. We observed cells that do not correspond to the printed type in two separate nanowells, one cell in each case (arrows).



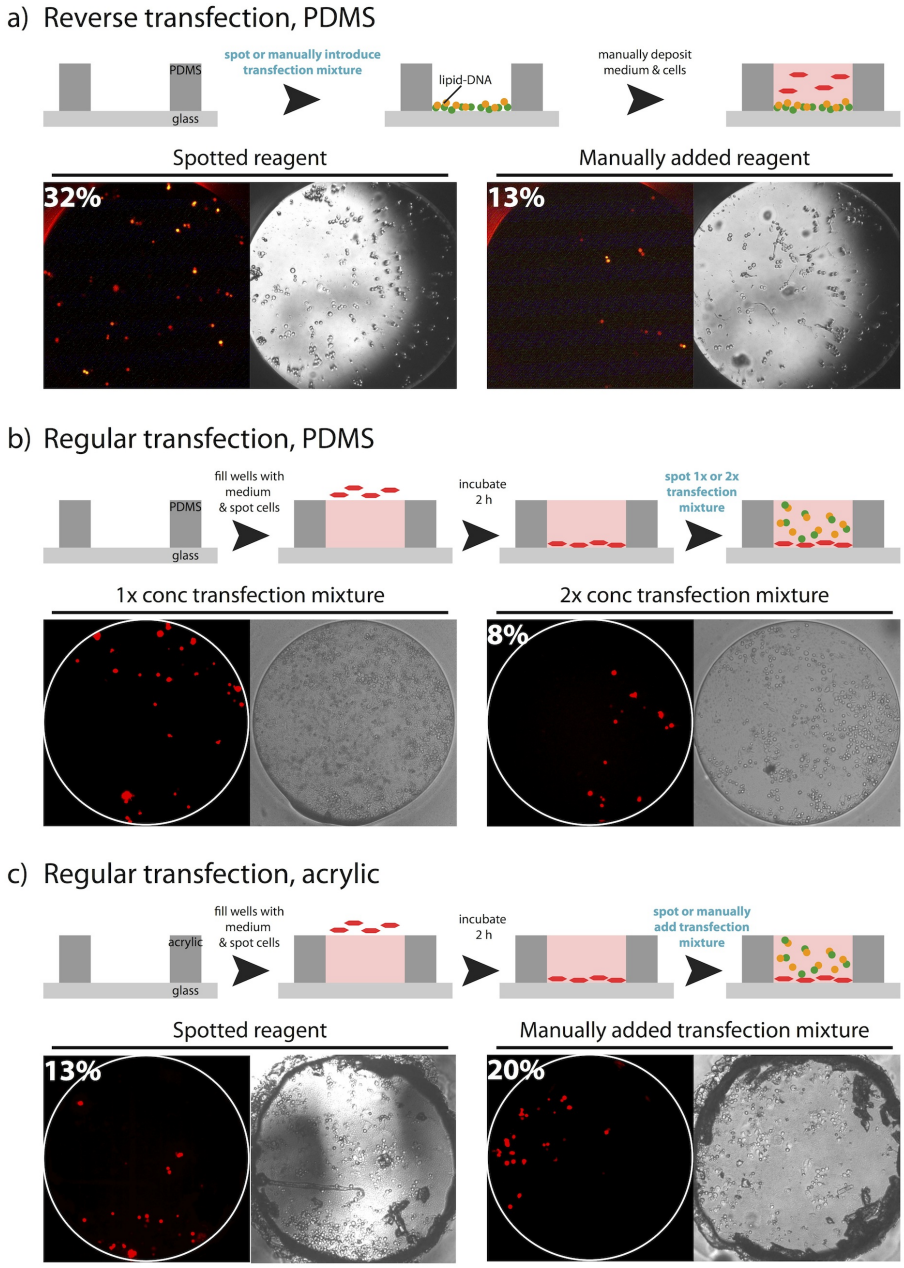
Supplementary Figure 4: Micrographs of four nanowells where an increasing number of cells were printed. The upper and bottom rows show cells after one and three days of culture, respectively. Cells grew rapidly, indicating that they sustained no damage during the spotting process.



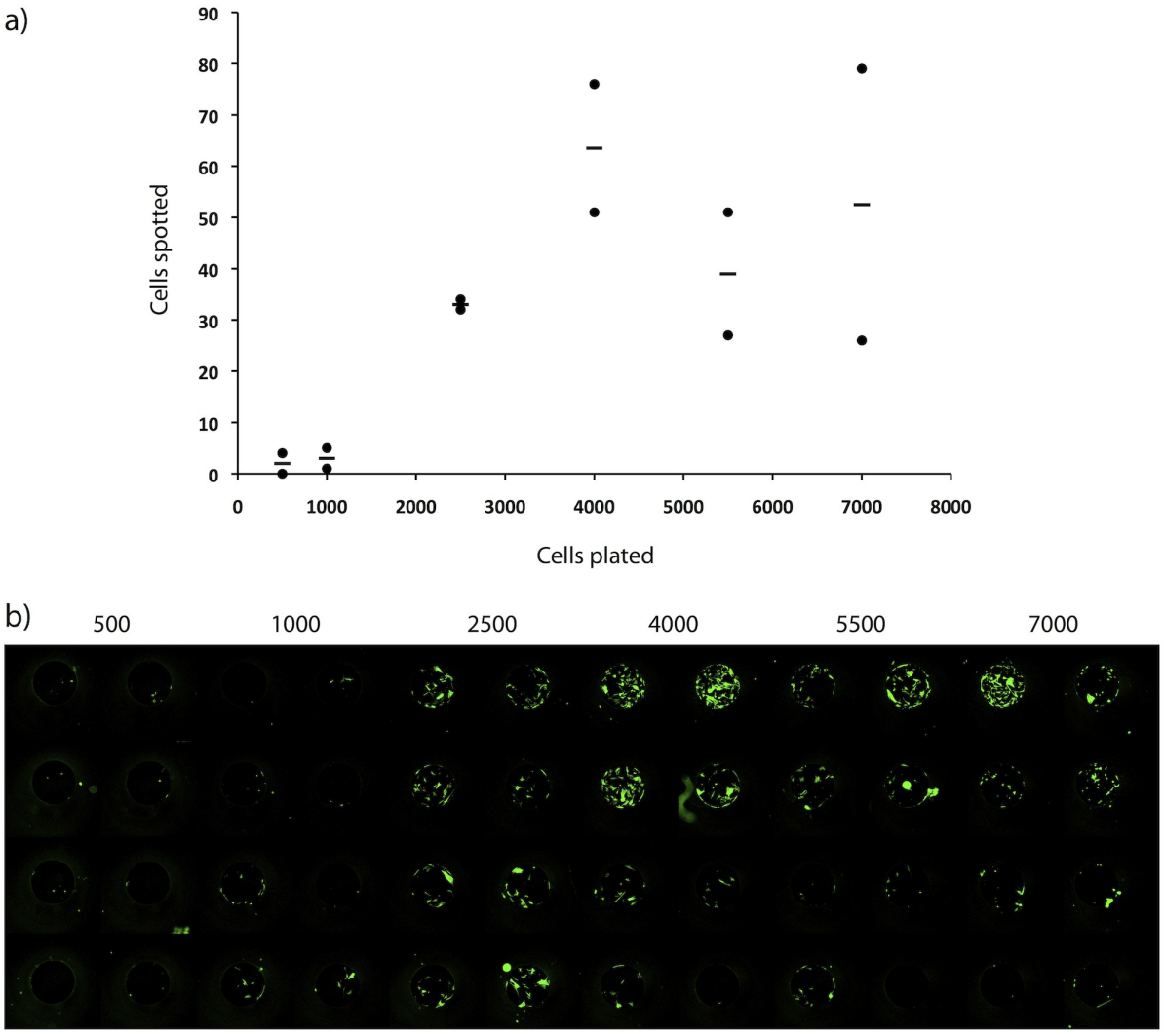
Supplementary Figure 5: Freezing of LMCAs. The cells used for the 48 well PDMS array were tomato and GFP NIH-3T3 fibroblasts (left two panels). Micrographs of the 48 well array were taken immediately before freezing and 3.5 h after thawing the array. CHO cells were used for the 675 well array (right two panels). Micrographs of the 675 well array were taken immediately before freezing and 22 h after thawing the array. Cells did not significantly detach from the array during the freeze/thaw procedure and remained viable.



Supplementary Figure 6: Collage of micrographs of an array featuring four different cell types separated by rows: NIH-3T3, HepG2, CHO and HEK293. All cells are viable after one day of culture, and those expressing a fluorescent marker continue to do so. For each row in the array, three separate input wells containing 10,000 cells each were used. Cells were printed sequentially from left to right.



Supplementary Figure 7: **(a)** Reverse transfection on the 48 well PDMS array. Lipid-DNA mixture was either spotted or manually deposited into the nanowells and allowed to dry, followed by manual addition of CHO cells to the array. Images were acquired after 20 h. The left panels show transfected cells expressing tomato, and the right panels show all cells in brightfield. When available, transfection efficiencies are indicated in the fluorescence images. **(b)** Regular transfection on the 48 well PDMS array. CHO cells were spotted into the array, followed by spotting of a 1x or 2x concentrated transfection mixture into the wells. Images were acquired after 42 h. Considerably lower efficiency was observed with the PDMS array when compared to the acrylic array. Using higher concentrations of transfection reagent did not improve efficiency. The hydrophobic PDMS may sequester the lipid transfection reagent; in regular transfection the lipid-DNA mixture is exposed to the PDMS when it is added to medium-filled nanowells. **(c)** Regular transfection on the 675 well acrylic array. CHO cells were spotted into the array, followed by spotting or manual deposition of transfection mixture into the wells. Images were acquired after 45 h.



Supplementary Figure 8: **(a)** Quantitation of the MSC array shown in (b). The number of hMSCs spotted into the array can be tuned by adjusting the number of cells in the well plate sample. Two separate well plate preparations were tested for each cell concentration (circles, with the mean value represented by the bar). hMSCs were spotted into the 48 well array at increasing densities from left to right. The number of cells in each sample well ranges from 500 to 7,000. Each sample in the well plate was used to spot 4 nanowells (one column), resulting in decreased cell density from the top to the bottom of each column. Only the top row of the spotted array was used for quantitation. **(b)** The hMSC cell array. Phalloidin staining was used to visualize cell shape.